THE SPORANGIUM OF THE OPHIOGLOSSALES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XCIV

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(WITH PLATES III AND IV)

The sporangium of the Ophioglossales has been studied by RUSSOW ('92), GOEBEL ('80, '81), HOLTZMAN ('92), ROSTOWZEW ('92), CAMPBELL ('95, :05), BOWER ('96), CARDIFF (:05), STEVENS (:05), and BEER (:06). Of these the last three concern themselves almost wholly with the later stages of the development of the sporangium and with the development of the spores up to the time of shedding. The former investigators devoted their principal attention to the early stages of sporangial development. Of their accounts, BOWER'S is the most comprehensive and, so far as the earlier stages of Ophioglossum are concerned, the most satisfactory.

In the fall of 1906 there came into my hands, through the courtesy of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, some fertile spikes of *Ophioglossum reticulatum*, which had been collected by Dr. H. N. WHITFORD at Lamao, Bataan, Philippine Islands. The spikes, from plants growing at an altitude of seventy-five feet above sea level, were collected July 26, 1904, killed and fixed in chrom-acetic acid, run up through the alcohols to 70 per cent., and sent to Chicago.

With the completion of this investigation each of the three genera of Ophioglossales will have been studied throughout the entire period of development of the sporangium and spores up to the time of shedding, so that a discussion of the development of the sporangium in the group seems timely. In this discussion Ophioglossum will be made the type, with frequent comparison with the other genera. The material of *O. reticulatum* showed no stages earlier than those represented in *figs.* 1 and 8, where the wall is about three-layered, the tapetum one-layered, and the sporogenous tissue about two divisions short of the mother-cell stage. This renders it necessary to obtain the account of earlier stages from the writings of former investigators.

Earlier stages

OPHIOGLOSSUM

According to BOWER, the first indication of the regions from which sporangia are to form is the differentiation along each side of the spike of a band about two cells wide. The cells of this band are distinguished from their neighbors by a greater depth, a difference in arrangement, and differences in cell contents. He calls this the sporangiogenic band. The cells are for the most part arranged in pairs, but he does not regard this as sufficient evidence of their common origin, for he says that if the cells concerned in forming a single sporangium originate from a single cell, it must be very early in the formation of the spike. Nor did he succeed in distinguishing them at a stage so early as to enable him to trace their origin. At a slightly earlier stage, GOEBEL figures the band as only in part two cells wide. His figure seems to support his contention that the band becomes two cells wide by the longitudinal anticlinal splitting of the cells of a band but one cell wide. Some of BOWER'S figures show a far less regular arrangement of the cells in the band. In these cases, as he clearly points out, it is much less easy to refer the origin of the band to a single row of cells. It is not unlikely that the difference of opinion is due to the recognition of the band at slightly different stages in the growth of the sporangial spike, for it is not unusual for subsequent growth to displace cells so as to render their relationship to one another obscure. Whatever may be the exact mode of origin of this band, the really important point is that sporangia may arise in any part of it, though in fact they do not arise in all parts of it. It is this partial sterilization of a band potentially sporangiogenic throughout on which BOWER lays special stress.

The cells of this sporangiogenic band divide periclinally, separating a potentially primary sporogenous band from the wall cells. According to BOWER, each archesporium consists usually of a single pair of the cells of the band; where the band is three cells wide, there are three cells in the archesporium. GOEBEL, with whom CAMPBELL agrees, thinks the archesporium is unicellular. ROSTOWZEW also claims a unicellular archesporium, but RUSSOW contends that it is a cell-complex. From such divergent opinions the truth is difficult to choose, though it seems not unlikely that the band may be recognizable

while only one cell wide, and that the first division in each cell is periclinal and cuts off the potentially sporogenous cells from the ones destined to form wall and tapetum. The outer segments, according to BOWER, by repeated division add considerably to the sporogenous tissue. It is specifically stated by BOWER and ROSTOWZEW, and implied by CAMPBELL, that the tapetum arises from the outer part of the sporogenous tissue.

The species studied were *O. pendulum*, *O. vulgatum*, *O. reticulatum*, by BOWER; *O. pendulum* by CAMPBELL; and *O. vulgatum* by GOEBEL and ROSTOWZEW.

HELMINTHOSTACHYS AND BOTRYCHIUM

In *Botrychium virginianum* HOLTZMAN figures the sporangium as arising from a single cell. Writing of Helminthostachys, BOWER says: "The sporangia are similar in origin and mature structure to those of Botrychium, the essential parts being referable to a single superficial cell, of which the first periclinal division defines the sporogenous from the protective parts." In agreement with RUSSOW he thinks that in at least some species of Botrychium the archesporium may consist of more than one cell. GOEBEL and CARDIFF think the archesporium in *B. lunaria* and in *B. virginianum* is one-celled. CAMPBELL likewise thinks that the archesporium of Botrychium is a single cell and that the entire sporangium is referable in origin to this one superficial cell. He ascribes the origin of the tapetum partly to the wall and partly to the outer sporogenous tissue. GOEBEL figures it arising from the wall and sterile cells surrounding the sporogenous tissue. CARDIFF also is positive that it comes wholly from the wall and tissue outside of the sporogenous mass. In Helminthostachys BOWER has shown that the sporangium originates from a single superficial cell, that there is a single primary sporogenous cell, and that the tapetum arises from the wall.

Later stages

The preceding account brings the development to the earliest stages shown in the writer's material, the discussion of which may be taken up conveniently under three heads: the wall, the tapetum, the sporogenous tissue.

THE WALL

Owing to the conflict of opinions, the youngest material at hand was examined for evidence of the origin of tapetum and sporogenous tissue. These sporangia have a wall three cells thick, or in some cases the spindle and beginning of the wall between the middle and innermost cell. *Fig. 1* shows the next stage, where the wall is three or four cells thick. Where the wall is four cells thick and the position of the walls indicates the last one formed (wall *1* in the figure), the division is seen to have occurred in the outer cell of the three-layered wall. The fifth layer of cells also arises in a similar way by the division of the cells of the outer layer of a four-layered wall (*figs. 2* and *3*, wall *1*). This is made clearer by an examination of the cell row between the two containing five each in *fig. 2*. It may be seen that the next division will occur in the outer cell.

The order of development may be stated in the reverse order as follows: If the cell layers be numbered from within outward *1*, *2*, *3*, *4*, *5*, then *5* and *4* arose from the division of a cell which we may call *c*. In like manner *c* and *3* arose from the division of a cell *b*. As already pointed out, *1* and *2* arose from the division of a cell *a*. The tapetum is already formed and differentiated at the two-layered stage of the wall, so that two suppositions may be entertained in regard to the early history of the wall. First, *a* and *b* may have been derived from the division of a cell *w*, sister to the tapetal cell. Second, cell *a* and the tapetal cell may be sister cells derived from the division of a cell sister to *b*. Of these two suppositions, both of which assume the tapetum to have originated from the wall, the former seems the more probable. That the tapetum does arise from the wall seems to be indicated by the close similarity of its cells in shape and position to the tabular wall cells and their contrast to the polygonal sporogenous cells. In the very earliest stages observed it is difficult to distinguish tapetal cells from wall cells except by position, though the sporogenous cells are easily distinguishable in almost all cases. It is not unlikely that the first division of the cell (or perhaps cells) of the sporangiogenic band is a periclinal one and separates the primary sporogenous from the primary wall cell. A periclinal division of the latter separates the primary tapetal cell from the wall cell, subsequent divisions of which produce the five-layered wall. If this interpreta-

tion be accepted, then Ophioglossum agrees with the other genera in the origin of sporangium, wall, primary sporogenous cell, and tapetum.

This regularity of division is limited to the part of the outer wall which from its position may be inferred to have arisen from the sporangiogenic band. In other parts of the wall, not only is the division less regular, but the number of layers is greater. Furthermore, longitudinal sections show that probably this regularity is still further limited to the cells derived from the initial cell (or cells) of the sporangium.

By the time the sporogenous tissue has reached the mother-cell stage, the wall has its full number of cell layers. The further development of the wall consists of an increase in size of the hypodermal cells in the tangential plane, without any increase of thickness, and a growth and anticlinal division of the epidermal cells. In mature sporangia the epidermal cells are relatively deep and narrow, with thickened outer walls. *Fig. 7*, which shows a sporangium at time of dehiscence, may be compared with *fig. 6*, from a sporangium whose sporogenous tissue had reached the stage of young tetrads. *Fig. 7* has just half the magnification of *fig. 6*, from which it is evident that the epidermal cells have just about doubled their depth, and have in some cases decreased in width through anticlinal division. As already remarked, the hypodermal cells do not increase in thickness, but become extended laterally as the sporangium enlarges. From about the time the young spores begin to round up, they gradually lose their cell contents and become flattened as growth of the sporangium progresses. Sooner or later the walls of the inner two or three layers, and often all the hypodermal layers, disappear. In *fig. 7* portions of the walls of two layers can be traced, but in other cases the only remains consist of a line of débris between the spore-mass and the epidermis.

At the earliest stage examined, the wall cells contain considerable starch, which increases up to the time the wall has attained its full number of cell layers. After this, which is about the time the tapetal plasmodium invades the crevices of the sporogenous tissue, it gradually wastes away, until none at all is present at time of dehiscence. Its disappearance may be connected with the accumulation of starch in the tapetal plasmodium, a matter which will be discussed subsequently.

Longitudinal sections (*figs. 6* and *7*) show the method of dehiscence. About the time the young tetrads are forming, each sporangium shows a slight transverse groove. *Fig. 6* shows that this corresponds to a band of smaller thin-walled cells. The groove becomes more pronounced through the failure of the epidermal cells along it to increase in depth as fast as the neighboring ones. Finally a rupture of the sporangium occurs along this groove, probably due to strain set up by unequal growth. The spores are shed through this slit, which, so far as the material at hand indicates, seems to play a purely passive rôle in the process.

THE TAPETUM

The youngest stage of the tapetum observed is shown in *fig. 1*. That this probably originates from the first periclinal division of the primary wall cell has already been pointed out. It divides periclinally once (sometimes oftener, as shown in *fig. 5*), before the cells of the sporogenous tissue have reached the mother-cell stage. Usually no more strictly periclinal divisions occur. As the sporangium grows, the tapetal cells elongate and divide obliquely (*figs. 2, 3, 5*). *Fig. 3* presents a regional view of about one-half of a sporangium with the tapetum well developed and distinct at the left of the figure, where it may be supposed to be derived from the primary wall cell. At the right of the figure the tapetal cells are not easily distinguished from the sporogenous tissue, and their origin is not clearly indicated, though they seem rather more closely related structurally to the sporogenous cells than to the sterile cells around them on the outside. In Botrychium GOEBEL has shown that this part of the tapetum arises from the sterile cells outside the archesporium. In the present study no clear evidence for either view was obtained, owing to lack of young sporangia.

At the earliest observed stage the tapetal cells are distinguishable from the sporogenous tissue by staining reactions of the cell contents and by the position of the walls. They are less easily distinguished from the wall cells, but are on the whole slightly thicker radially. The cytoplasm is clear and highly vacuolate and so takes the stain lightly. It does not contain starch nor any form of stored food evident under the microscope. In this respect it differs from the

cytoplasm of the inner wall cells, which even at this stage contain a little starch; the outer wall cells contain considerable at this time. The tapetal cytoplasm was not observed to contain any starch until after the walls have broken down. The walls themselves are from the first very delicate, but are sharp and distinct when properly stained, so that there can be no mistake as to their presence. In this respect Ophioglossum seems to differ from *B. virginianum*, whose tapetal cells, as described by STEVENS, are delimited by a plasmatic membrane only. CARDIFF, who studied the same species, does not mention this circumstance, and BEER calls attention to the fact that it is not true in Helminthostachys. The nuclei are about 20 μ in diameter, but vary somewhat both in size and shape. They stain rather deeply and are generally not otherwise distinguishable from the nuclei of the wall cells.

When the mother-cell stage is reached, the tapetum is at least two-layered throughout and its walls have become very thin and about this time have begun to break down. The cytoplasm of the neighboring tapetal cells mingles and forms a plasmodium, as described by CARDIFF for Botrychium. In Botrychium as well as in Helminthostachys the tapetal cells are elongated radially, while in Ophioglossum they are flat and tabular, the radial diameter being the shortest. Ophioglossum also differs from the other genera in having usually only two layers of tapetal cells, whereas they have from two to four layers. It seems likely that the walls break down sooner in Ophioglossum than in the other genera. This is probably correlated with the fewer layers and with the fact that in Botrychium the cells are frequently binucleate. Too few preparations at just the stage when the walls are breaking down were seen to make sure that binucleate cells do not occur, but none were seen.

BOWER and others have stated that at this time some of the mother cells break down and contribute to the tapetal plasmodium. This is denied by CARDIFF for Botrychium, and by BEER for Helminthostachys. No certain case of such a contribution was observed in Ophioglossum, but the number of preparations of just this age observed were too few to justify the statement that it never occurs. Mother cells which did not appear normal were found in two or three preparations, but in each case they occurred in sporangia that were

in other respects clearly abnormal, suffering from external injury to the wall in one case, an abortion of the inner wall cells and neighboring sporogenous tissue in another, etc. In sporangia apparently normal in all respects, a few of which were observed, no trace of such a contribution was found. It is not unlikely, therefore, that this genus agrees with the other two in this feature also.

At the time the plasmodium begins to penetrate among the crevices of the sporogenous tissue they are rather small, and the movement is therefore gradual. Hence the deeper parts of the crevices are empty of cytoplasm. By the time the sporangium has reached the stage shown in *fig. 4*, the plasmodium completely fills every crevice, showing that the cytoplasm increases in volume after the breaking-down of the walls. The nuclei do not at first penetrate among the blocks of sporogenous tissue on account of the narrowness of the clefts, but later as these enlarge they become distributed throughout the sporangium.

The structure of the plasmodial cytoplasm is at first coarser and stains more deeply than did that of the tapetal cells before the breaking-down of the walls. It has a ropy appearance and contains many large granules. It does not seem to be definitely vacuolate, but among the beaded threads there seems to be a fine hyaline sap-like substance (*fig. 4*). At this time the plasmodium does not contain any starch, which, however, soon appears and continues to increase until the plasmodium is fairly crowded with it (*fig. 16*). The stringy cytoplasm becomes denser during the formation of the starch and finely vacuolate. The maximum density and starch content is reached about the time the tetrads are complete. From this time on the starch grains become fewer and fewer (*fig. 25*) until they have entirely disappeared. By this time the cytoplasm, which has been growing more and more vacuolate, has nearly all disappeared. The last traces of it are to be seen along the old plasmatic membranes, which may often be traced long after the more fluid parts have disappeared. When the spores are of the age shown in *figs. 26* and *27* all traces of it are usually lost.

The nuclei of the plasmodium exhibit frequent changes of form in order to accommodate themselves to the spaces which they are compelled to occupy, or into which they perhaps force themselves. Where one is found in a narrow cleft it has the appearance of being about to

divide amitotically. No actual case of such amitosis, however, has been observed in any stage of the tapetum. No mitotic figures have been observed in the plasmodium either, though the nuclei seem somewhat more abundant in the later stages. The sporangia vary so much in size and consequently in number of nuclei found at any stage that it is not possible to say with confidence that the nuclei increase after the walls of the tapetum have disappeared. That the nuclei do not move about passively in currents of cytoplasm seems to be indicated by the fact that where one is apparently just entering a crevice it is always drawn out to a point, which stains more deeply with chromatin stains than do the other parts of the nucleus. BEER has called attention to similar appearances of the nuclei of Helminthostachys. The average size of the nuclei does not differ materially in older stages from that of the earlier ones. There is considerable individual variation of both size and shape at any stage. CARDIFF has pointed out that the nuclei of the plasmodium of Botrychium increase fourfold in size; but BEER has found that this is not the case in Helminthostachys. The structure of the nucleus, however, does undergo considerable change. A reference to *fig. 16* shows the tendency of the chromatic material in the later phases of development to aggregate itself into elongated masses, whose long axes are directed lengthwise of the nuclei. These masses are much fewer than the chromosomes. A similar condition is shown in BEER'S figures of Helminthostachys. When the nuclear membrane breaks down, these masses are spilled out and persist for some time. The nuclei persist longer than the cytoplasm; it is not uncommon to find one or more of them in sporangia from which the spores are just about ready to be shed.

THE SPOROGENOUS TISSUE

The earliest stage of the sporogenous tissue observed is shown in *fig. 8*. At this time it probably lacks about two divisions of the mother-cell stage. The cells are squarish or polygonal, and about 25 μ in diameter. The nuclei, which vary slightly, average about 18 μ in diameter. The cytoplasm is delicately reticulated and does not stain very intensely. It seems to be made up of a network of delicately beaded strands, the meshes of which are filled with a hyaline ground-substance which stains very lightly or not at all. The

wall is very thin but distinct. STEVENS reports the sporogenous cells of Botrychium as being devoid of a wall. The cells at this stage, in the living condition, are probably still attached to one another so as to form a compact tissue. In stained preparations clefts appear among groups of cells. It is probable that some or all of these clefts are the effects of contraction in the preparation of the material. The nucleus is usually quite round and is surrounded by a sharply defined nuclear membrane. The nuclear network is not very unlike that of the cytoplasm, except that the beaded strands are larger, stain more deeply, and present many more loose ends. From one to six nucleoli are usually present in each nucleus.

Whether the blocking of the sporogenous tissue begins as early as the stage shown in *fig. 8* or not, there is no doubt that it is clearly present after the next division of the sporogenous cells. Such a condition is shown in *fig. 2*, where the sporogenous tissue is seen to be broken up into irregular blocks, and to be free from the tapetal cells, which are beginning to separate from one another. *Fig. 3* presents a view of a slightly more advanced state of blocking. The irregularity of Ophioglossum is in sharp contrast to the almost diagrammatically regular blocking of the sporogenous tissue of Botrychium. According to CARDIFF, the division of the sporogenous tissue in that genus is in three planes at right angles, and very regular and uniform in all parts of the sporangium. He suggests that these walls break down in the order of their formation, with the result that the tissue breaks up into regular rectangular blocks. His figures lend considerable support to this view, and there seems to be no reason for doubting it. The divisions in the sporogenous tissue of Ophioglossum are far less regular, and this may be the cause of the irregularity in the blocking. BEER notes that the process in Helminthostachys is less regular than in Botrychium. It is far more regular, however, than in Ophioglossum. An exceptionally regular case is shown in *fig. 5*.

Correlated with the question of blocking is that of the division of the sporogenous cells. In Botrychium, and to a less degree in Helminthostachys, division is simultaneous in the sporogenous tissue up to the time of blocking. After the blocks become independent, the cells of each block divide simultaneously, but different blocks may develop at different rates. Even so early as the stage shown

in *figs.* 1 and 8, the cells do not develop at the same rate nor do they divide simultaneously. That there is some coordination, however, is shown by the fact that figures do not usually occur isolated, but in small groups. Cells in the synapsis stage were noted in some sporangia when other cells seemed not to have reached the mother-cell stage. Oddly enough, however, most or all of the cells come out of the synapsis at about the same time. This seems to be the only point in the development at which the cells are all at approximately the same stage. Each cell seems to develop independently of the others in the majority of cases, although there are not lacking frequent cases where several cells lying side by side are approximately in the same phase. The most striking examples of this, are groups of cells in the late prophase often found among young tetrads or even in sporangia, where the young spores have become separated and have rounded off. *Fig.* 9 gives a view of a mother cell in synapsis (the synaptic knot has been simplified in drawing for the sake of clearness). The cells at this stage are oblong and about $28 \times 32\ \mu$. The cell shown in the drawing is one of the largest, being $27 \times 40\ \mu$. The walls are at this time very thin and the cells have either separated from one another entirely or into small groups. The walls are so thin that it was sometimes impossible to find them, though it was always possible to find other cells younger, of the same age, and older, which showed the wall, so that it seems likely that all of them really have walls. It has already been noted that STEVENS did not find them in *B. virginianum* at certain stages. CARDIFF shows in his figures what seem to be walls, but makes no text comment. BEER is emphatic in his assertion that they are present at all stages in Helminthostachys. Recalling their exceeding thinness and the difficulty of staining them, one must accept with considerable reserve the statement that they are absent at any stage. However, when one considers that most of the walls disappear in the breaking-up of the sporogenous tissue, it does not seem altogether improbable that they may totally disappear in some cases. It may be added that the writer has been able to find them in preparations of *B. virginianum* of the same age. The preparations examined do not cover all stages, and he is therefore not able to say positively that they are always present.

The cytoplasm is very like that already described for the earlier phases of development, except that it is somewhat denser, especially around the nucleus. The synaptic knot is usually so closely massed that the course of the individual thread or threads cannot be followed for any great distance. The figure was drawn from one of the most diffuse knots in a section 10 μ thick. The chromatin seems to consist of a single row of granules strung along a continuous much tangled thread. The thread could not be determined to be continuous by direct observation, but this is made probable by the fact that there are no loose ends protruding from the knot on the uncut side. The same is true of other knots. In the early stages the thread is extraordinarily long and thin, long enough nearly to fill the nucleus with its tangled folds, and not more than 0.25–0.5 μ thick. The thread breaks up into very long slender chromosomes, which may have almost any shape from nearly straight to intricately bent and twisted.

A somewhat later stage is shown in *fig. 10*, where the chromosomes have shortened by one-half or more and have become correspondingly thicker. In the figure no attempt has been made to represent all the chromosomes in the nucleus, but only those whose shape and position could be made out clearly. The drawing was made from a section in which the knife has passed almost exactly through the center of the nucleus, and the cut side of the nucleus lies up. This gives a clearer field, but has the disadvantage that some of the chromosomes are cut in two. More than sixty chromosomes are shown in the drawing of this half-nucleus. Attempts were made to count the chromosomes at this stage, in the stage shown in the next figure, in polar views of the second division, and elsewhere, but without success. The number exceeds 100, but probably does not reach 120 for the gametophytic tissues. The chromosomes continue shortening and thickening until they reach the shape and size shown in *fig. 11*. The nuclei have their maximum size at about this stage, being 23–24 μ in diameter.

During the development of the chromosomes the wall remains thin and stains lightly, as in previous stages. The cytoplasm, however, has increased in density somewhat and has become more finely vacuolate. There appear in it about this time certain oval bodies staining very intensely with Haidenhain's iron-alum and haematoxylon, but

lightly with most other stains. The staining reactions make it improbable that they are starch or chromatic material or any of the more usual constituents of cells. They are probably some sort of stored-food material, since they disappear slowly at a later stage, when such stored foods are being used. They appear in all stages following this up to about that shown in *fig. 23*. Their number and size in different cells varies greatly, in some being absent. Some of them are shown in *figs. 11–21*. They are usually much more numerous than shown in the figures, which show only those visible in a single optical focus, or even fewer than that in *figs. 17–20*. They often line up along the central region of the spindle of the first division, or along the subsequent wall (when one forms) in such masses as to obscure the details of cell structure entirely in that region. The cytoplasm itself grows denser and denser as the time of division approaches; *fig. 11* shows it at about its maximum. *Figs. 11* and *13* show two different aspects of the cytoplasm of dividing cells, the former being the common one. The beaded threads shown in *fig. 12* are very characteristic of the cytoplasm of the cells during maiosis.

The spindle originates as a weft of kinoplasmic fibers around the nucleus. The fibers then form a number of poles such as those shown in *fig. 11*. Three of these poles become prominent and the others disappear. The tripolar stage passes into the bipolar by the further elongation of two poles and the breaking-up of the third (*fig. 12*). *Fig. 13* presents a surface view of the mature, sharply pointed, bipolar spindle. The chromatic mass (simplified in the figure) proved too dense and the chromosomes too small and numerous to make any trustworthy observations on the method of chromosome splitting. The chromosomes usually pass to the poles in a more or less straggling manner, though sometimes they advance in regular lines. Reaching the poles they may or may not organize a resting nucleus; when the second division follows very soon they probably do not. *Fig. 14* shows the beginning of the formation of the resting network before the spindle has disappeared. *Fig. 15* shows the chromosomes still free after the formation of a wall between the daughter nuclei. In *fig. 16*, from which the spindle fibers have not entirely vanished, the daughter nuclei are in the resting condition.

After the first division, a wall may (*figs. 15, 16, 19, 20*) or may

not (*figs. 14, 17, 18, 21*) form before the next division. Probably this depends on the rapidity with which the second division follows the first, and perhaps the usual thing is for the second division to occur when the daughter nuclei have reached about the stage shown in *fig. 14*. At this time the chromosomes usually have not formed a resting network, and the central part of the first spindle still persists. If division occurs at this stage, the resulting figure usually resembles *figs. 17* and *21*. The majority of the spores are of the tetrahedral type. In this region of the cell, whether the wall forms or not, there is always a noticeable collection of fine granular cytoplasm which is sharply distinguishable from the less dense parts containing the beaded strands already mentioned. Within this zone most or all of the food granules are found; they are often so numerous and so densely crowded as to make it difficult or impossible to tell whether a wall is present or not.

Early stages in the development of the spindle of the second division were not observed. Metaphases are shown in *figs. 17* and *18*, and anaphases in *figs. 19* and *20*. All the spindles with the exception of the type shown in *fig. 18* are sharply bipolar, though somewhat blunter than those of the first division. The spindle fibers are markedly different from those of the first division; instead of a loose bundle of very fine fibers, the fibers are heavy and coarsely beaded. All the spindles arranged as in *fig. 18* have their apices truncated. *Fig. 19* shows one spindle in the linear position, the other not; in this type no truncated spindles were observed. *Fig. 20* shows the bilateral arrangement; no other figure with this arrangement was observed, though one case of bilateral spores was noted (*fig. 25*). *Figs. 17–20* are arranged in the order of frequency of their occurrence in the preparations. No spores were found which could be referred to the types of division shown in *figs. 18* and *19*, which is singular, considering the frequency of this type of arrangement at time of division; they were probably present, but were not recognized. In *fig. 20* the chromosomes seem to be passing into the network characteristic of resting nuclei even before reaching the poles of the spindle, and long before any nuclear membrane has formed.

During maiosis the wall of the mother cell still persists and is very noticeable, owing to its separation from the contents, for which it

seems ordinarily to be far too large. Such a wall, in contact with the contents at one point only, is shown in *fig. 15*. This wall persists around the tetrad until late in the development of the spores. The wall with its contents lies in a vacuole of the tapetal cytoplasm. The vacuole is limited by a plasmatic membrane often more prominent than the wall of the mother cell within it. Where the two are in contact they are distinct, showing that the vacuole is a real one and is not merely the effect of shrinkage in preparing the material. I was not able to determine just how long the old wall persists, but it was found (at least parts of it) around spores as old as those shown in *fig. 27*.

The beginning of the tetrad walls is shown in *fig. 21* and the completed wall in *fig. 22*. It is formed partly on the primary division spindles of the second division and partly on secondary spindle-like structures which arise between the nuclei which are not sisters. Of the six walls which meet in the middle and separate the young spores, two form on the primary spindles and four on the secondary ones. At the same time a wall forms on the outer or convex side of the spores. Whether this wall joins directly to the tetrad wall or continues around inside of it could not be determined. BEER describes this tetrad wall as uniting with the wall which surrounds the tetrad, thus forming a structure with four compartments, each of which contains a spore. It is certain that it does not do so in Ophioglossum, owing to the fact that the mother-cell wall is not at that time in contact with the spores.

The cytoplasm of the young spores is dense and granular. The resting nucleus has a rather coarse network, with the chromatin scattered over it in rather large masses (*fig. 22*). As the spores grow older they break apart, the walls thicken, and the cytoplasm becomes vacuolate. The progressive vacuolation of the cytoplasm seems to be connected with the corresponding rapid thickening of the young spore coats. The relation between the two is brought out in *figs. 22–26*. During this period of thickening of the spore walls the cytoplasm of the tapetal plasmodium also becomes more vacuolate. A decrease of its starch accompanies *pari passu* the vacuolation of the plasmodium. The degree of the collapsing action of the killing and fixing agents on the young spores affords a graphic though not very

accurate means of estimating the decreasing density of their cytoplasm. At about the time the spore coats attain a sufficient rigidity to resist collapse, the cytoplasm begins to increase in density (*fig. 27*). Soon afterward (*fig. 28*) starch makes its appearance in the spore. About the same time the spore coat can be seen to consist of an exospore and an endospore. As the spore becomes older the two regions of the wall become more distinct and the exospore increases considerably in thickness. In the mature spore there are two cytoplasmic zones, an outer less dense one and an inner dense one filled with starch.

Whether the endospore originates as a separate membrane or whether it is merely the inner part of an originally homogeneous membrane which has differentiated into two could not be determined in Ophioglossum. Appearances, however, favor the latter view. Perhaps the strongest evidence in its favor is the fact that though the endospore is less distinct when first distinguishable, it is just as thick or even thicker than in the mature spore. Somewhat various opinions have been held concerning the exact method of exospore formation. DEBARY (**'64**) states that the exospore of certain Ascomycetes is laid down from the epiplasm, which might be thought to correspond roughly with the tapetal plasmodium in respect to the part either might play in the formation of the outer coat of the spore. The fact that the entire tetrad is surrounded for a considerable time by the persistent mother-cell wall, and that the whole lies in a vacuole, would seem to render this hypothesis untenable in respect to Ophioglossum. FITTING(**:00**), BEER (**:04**), and others maintain that the exospore, even though separated from the endospore and the protoplast by a space containing no protoplasm, can be built up by its activities. Miss LYON (**:05**) has shown that the two coats in Selaginella are formed by the differentiation of a single thick homogeneous gelatinous membrane. The exospore according to this view is simply the outer part in which has been deposited dense granular material; the roughness is due to the irregular way in which the material is deposited; and the endospore is the part in which no such deposits are made. This explanation seems to harmonize with the facts in Ophioglossum. Miss LYON points out that the membranes of Selaginella are not separated and suggests that where they seem so it is due to action of the reagents used in preparing the material. There is

no separation in Ophioglossum, and in consequence no necessity for action at a distance greater than the thickness of the spore coat. The supposed semi-fluid character of the membrane at the time of its formation would reduce this difficulty to a minimum.

Interesting irregularities occur in the development of the sporogenous tissue. It has already been pointed out that the mother cells may develop at very different rates. *Fig. 11*, for example, was drawn from a sporangium containing young tetrads and various stages of the first and second divisions. Still more interesting irregularities are shown in *figs. 30–33*. *Fig. 30* shows an enlarged mother cell in the metaphase of the first division; it was found among spores slightly younger than the one shown in *fig. 27*, and its wall is indistinguishable from those of the spores among which it lies. This figure afforded the best opportunity observed for obtaining an approximate count of the chromosomes. Owing to the way in which they are scattered, most of them are sufficiently free from their neighbors to be identified and counted; 102 are shown in the drawing, which represents about one-half the entire figure. About as many more could be counted in the sections adjacent to this on each side, thus making the gametophyte number somewhat over 100. *Fig. 33* shows three spores within a common exospore, one of which is plainly degenerating; no trace of the fourth member of the tetrad could be found. Such a structure could arise from the one just discussed by the division of one of the daughter nuclei and each of the three resulting nuclei and its accompanying cytoplasm surrounding itself with a wall. *Fig. 31* shows a body consisting of eight cells, with thin outer wall and very delicate division walls. The cytoplasm is dense, finely reticulated, and crowded with starch, which is also abundant in the tapetal plasmodium around it, though not found in the young spores in the same sporangium nor in any spores until they are much nearer maturity. This might suggest that the normal young spores are poor in cytoplasm and stored food because they use it to build their rapidly thickening walls. Though varying in size the nuclei are all large, being about the size of the entire young spores in the same sporangium. With Flemming's triple stain they stain uniformly with the gentian. *Fig. 32* represents another structure of the same sort but with twenty nuclei, two of which are very large. These bodies have probably arisen from

the germination of the members of a tetrad which failed to separate in the usual way. Possibly the lack of a thick wall around the young tetrad is responsible for the continued growth; at any rate, no indication of the germination of ordinary thick-walled spores was observed, unless *fig. 30* be interpreted as such. Not unlike this is the production of more than four microspores in some angiosperms, but so far as I am aware similar phenomena have been reported in only one homosporous plant. In an account of the germination of the oospore of Coleochaete, ALLEN shows that reduction takes place with the first division; the spores thus produced germinate and cooperate to produce a multicellular mass of gametophytic tissue. Each cell of this mass of tissue eventually produces a zoospore which gives rise to the ordinary vegetative phase of the plant. WILLE has shown (**'86**) that in certain angiosperms by a suppression of the second maiotic division a mother cell may give rise to two microspores only instead of the usual four. Since in at least the majority of plants the unlike elements of the bivalent chromosomes are separated in the first division of the mother cell, there seems to the writer no valid reason, other than that imposed by the available food supply and the ordinarily thick walls, why any number of spores more than two may not arise from a mother cell. In a discussion of many-spored asci OVERTON (**:06**) has recently shown that the number of divisions in the ascus is immaterial so far as the essential nature of the resulting spores is concerned. In this connection the recent experimental work of NĚMEC (**:06**) is of considerable interest; by subjecting staminate flowers to the action of chloroform vapor he secured pathological conditions closely paralleling those herein described. His attempts to germinate these bodies should yield interesting results.

SUMMARY AND COMPARISON

The following tabular form of statement will serve to contrast compactly the three genera of Ophioglossales as to their sporangia.

Ophioglossum	*Helminthostachys*	*Botrychium*
1. Outer wall and corresponding parts of the tapetum and the sporogenous tissue from one or two superficial cells of a sporangi-	The essential parts from a single superficial cell.	Like preceding but smaller contribution of neighboring cells.

	Ophioglossum	*Helminthostachys*	*Botrychium*
	ogenic band; remainder of sporangium formed by the cells of the adjacent sterile tissue.		
2.	Freely exposed outer wall 5-layered and from primary wall cell (or cells).	Similar to the preceding in origin and development, except that in most species the entire wall so arises.	Like preceding but still further restricted to a single cell in origin.
3.	Sporangia sunken.	Sporangia intermediate.	Sporangia generally short-stalked.
4.	Dehiscence by transverse slit.	By longitudinal slit.	By transverse slit.
5.	One to three primary sporogenous cells.	One primary sporogenous cell.	One primary sporogenous cell.
6.	Tapetum unusually two-layered and its cells tangentially elongated.	Cells radially elongated, otherwise like preceding.	Same as preceding except that there are two to four layers of radially elongated cells.
7.	Nuclei of tapetal plasmodium probably do not increase in number.	Amitosis of these nuclei probably occurs.	Amitosis usual and frequent.
8.	Nuclei of plasmodium do not increase in size.	No increase in size.	Nuclei increase to four times their original size.
9.	Tapetal nuclei persist up to near time of dehiscence.	More persistent than in preceding.	Less persistent than other genera.
10.	Sporogenous tissue separates into irregular masses.	Blocking much more regular.	Blocking extremely regular.
11.	Division of sporogenous tissue up to mother cells not wholly simultaneous.		Simultaneous division the rule.
12.	Maiosis in different mother cells varies widely in time and in arrangement of spindles.	Maiosis in each mother-cell independent.	Division simultaneous in each block of cells.
13.	Spindle extra-nuclear.		Spindle intra-nuclear.
14.	Chromosomes 100 to 120 after reduction.	Reduced number 40 to 60.	No data.
15.	Spindles in all possible positions with reference to one another.		"Either in same plane or perpendicular to one another."

Ophioglossum	*Helminthostachys*	*Botrychium*
16. Wall formed or not after first maiotic division.	Special tetrad wall inclоses each spore in a separate compartment.	No wall formed.
17. Tetrads for a long time inclosed within persistent mother-cell wall.		Tetrad inclosed within persistent wall of mother cell.

The features common to the genera are: (1) the breaking-down of the inner layers of the wall, (2) the penetration of a plasmodium, derived from the tapetal cells, among the sporogenous cells, (3) the failure of the mother cells to contribute to the tapetal plasmodium, (4) the formation of a resting nucleus after the first maiotic division, (5) the appearance in the spore-plasm of a phase of decreasing density followed by one of increasing density, the former possibly connected with the rapid growth of the young spore coat (no data from Botrychium), (6) the spores rich in starch, (7) the tetrads in vacuoles of the plasmodium.

This investigation was carried on under the direction of Professor JOHN M. COULTER and Professor CHARLES J. CHAMBERLAIN.

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EXPLANATION OF PLATES III AND IV

All figures were drawn with the aid of a Bausch and Lomb camera and Zeiss apochromatic objectives and compensating oculars, and reduced one-half. *Figs. 1–7* and *fig. 32* were drawn under a low-power dry lens, the others under 1.5^{mm} immersion.

PLATE III

FIG. 1. Wall of three or four layers; last wall formed indicated by *1;* tapetum one-layered except at left. ×300.

FIG. 2. Wall four- or five-layered; tapetum two-layered; sporogenous tissue beginning to break up into irregular blocks and to separate from the tapetum. ×300.

FIG. 3. General view of about one-half of a sporangium, showing four or five layers in the wall; two-layered tapetum; sporogenous tissue broken up into irregular blocks; the regular two-layered tapetum is restricted to the left of the figure; in the remaining part it is difficult to distinguish it from the sporogenous tissue; *1* is last wall formed. ×123.

FIG. 4. Tapetal plasmodium penetrating among blocks of sporogenous tissue; tapetal nuclei mainly around periphery; blocks of sporogenous tissue quite irregular. ×300.

FIG. 5. An unusually thick tapetum and more regular blocks of sporogenous cells, somewhat older than those of *fig. 4*. ×300.

FIG. 6. Longitudinal section of wall; tetrad stage of sporogenous tissue; to show early indications of formation of slit for dehiscence. ×300.

FIG. 7. Mature wall showing longitudinal section through line of dehiscence; inner cell layers broken down. ×123.

FIG. 8. A sporogenous cell lacking about two divisions of the mother cell; from sporangium with a three-layered wall. ×900.

FIG. 9. Synapsis of mother cell; synaptic knot has been simplified in drawing for the sake of clearness. ×900.

FIG. 10. Prophase of first division; chromosomes about one-half the length of their earliest stage; food granules in cytoplasm. ×1200.

FIG. 11. Origin of spindle; chromosomes in mature form, many of them seem paired; food granules in cytoplasm; cell appears small because it is not drawn in median optical section. ×900.

FIG. 12. Spindle passing from tripolar to the bipolar stage; food granules and beaded kinoplasmic threads prominent. ×900.

FIG. 13. Surface view of mature spindle at metaphase; cytoplasm unusually free from food granules and kinoplasmic threads. ×900.

FIG. 14. Telophase of first division; nuclear membrane formed and chromosomes mostly free from one another; middle region of cell occupied by persistent spindle fibers, food granules, and denser cytoplasm; old wall becoming freed from contents. ×900.

FIG. 15. Later telophase; a wall has formed, along which the food granules tend to line up; mother-cell wall almost entirely free from contents; chromosomes quite free from one another. ×900.

FIG. 16. Part of tapetal plasmodium containing much starch; tapetal nuclei show the aggregation of chromatic material into elongated masses, far fewer than the chromosomes; mother cell after first division, with a wall and daughter nuclei in resting condition; the whole lies in a vacuole of the plasmodium; old mother-cell wall surrounds it, but is not in contact with it and is not shown in the drawing. ×900.

PLATE IV

FIG. 17. Usual position of spindle with no wall between, will result in tetrahedral arrangement of spores; note difference between first and second spindles; many chromosomes left out for clearness. ×900.

FIG. 18. Linear arrangement of spindle; spindle fibers do not come to a point; as in all cases, granular cytoplasm and food granules collect in middle region, whether a wall is present or not. ×900.

FIG. 19. Combination of linear and bilateral arrangement; linear spindle pointed, though this was never observed where both spindles are placed linearly; a wall in this and next figure. ×900.

FIG. 20. Bilateral arrangement of spore nuclei; chromosomes already fusing before reaching the poles. ×900.

FIG. 21. Telophase and earliest indication of tetrad walls. ×900.

FIG. 22. Young tetrad with dense cytoplasm and thin wall. ×900.

FIGS. 23, 24. Progressive vacuolation of spore-plasm and correlated thickening of spore wall. ×900.

FIG. 25. Depletion of tapetal plasmodium and increase of its vacuoles; bilateral spores shown within vacuole. ×900.

FIG. 26. Maximum depletion of spore-plasm; spore walls rather thick and probably gelatinous, collapsing under the action of the reagents. ×900.

FIG. 27. Spore wall rigid enough to resist collapsing action of reagents; wall beginning to be slightly denser and rougher. ×900.

FIG. 28. Spore coat distinctly differentiated into two layers; the cytoplasm much denser and showing very small starch grains. ×900.

FIG. 29. Mature spore with thick wall and abundant contents; starch very abundant; intine penetrates exine at tip of spore. ×900.

FIG. 30. Much delayed division of a mother cell, spore-coat-like wall surrounding it. ×800.

FIGS. 31, 32. Multicellular gametophytic (presumably) masses of cells, which have arisen through the cooperative germination of the members of a tetrad which failed to separate in the ordinary way. ×233.

FIG. 33. Three of the four spores of a tetrad within a common exospore. ×600